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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
CASSELS, et al.	Art Unit: 1651
Serial No.: 09/942,974	Examiner: GITOMER, Ralph J.
Filed: 31 August 2001	Atty. Docket: 034047.0342 (WRAIR 97-30B)
For: MASS SPECTROMETRY OF COLONIZATION FACTORS	

DECLARATION OF RYAN T. RANALLO, Ph.D.
UNDER 37 C.F.R. 1.132

1. I, Ryan T. Ranallo, am a citizen of the United States of America and I reside at 8326 Tea Rose Drive, Gaithersburg, MD 20879.

2. My curriculum vitae is attached.

3. I have experience with and knowledge of mass spectrometry techniques including matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF) of proteins.

4. I have reviewed, analyzed, and understand the disclosure of the above-referenced patent application, U.S. Patent Application Serial No. 09/942,974 ('974 application), filed 31 August 2001.

5. I have reviewed, analyzed, and understand the Office action mailed 11 February 2004, in the above-referenced application.

6. I have reviewed, analyzed, and understand the abstract for ASM General Meeting entitled "Absolute Molecular Weight Determination of E. coli Fimbrial Major Subunits" (1993) by Cassels et al. attached herewith.

7. The abstract above does not teach or disclose that colonization factors should first be dissolved in hexafluoropropanol and to which acetic acid is then added second.

8. Attached herewith are two mass spectrographs. The first is of a colonization factor first dissolved in acetic acid and then hexafluoropropanol added second. The second is of a colonization factor first dissolved in hexafluoropropanol and then acetic acid added second.

9. The first mass spectrograph shows that dissolving the colonization factor in acetic acid first does not provide an observable signal of the 15055 and 15877 protein peaks.

10. The second mass spectrograph shows that dissolving the colonization factor in hexafluoropropanol first provides observable signals of the 15055 and 15877 protein peaks.

11. In my opinion, the 15055 and 15877 protein peaks are necessary for correctly identifying colonization factors, such as CS6, via mass spectrometry.

12. In my opinion, the abstract does not teach or disclose that colonization factors should first be dissolved in hexafluoropropanol and to which acetic acid is then added.

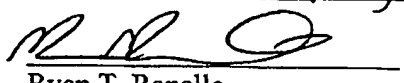
13. In my opinion, the abstract does not teach the criticality of first, solubilizing the colonization factor by dissolving the colonization factor in 1,1,1,3,3,3-hexafluoro-2-propanol and then second, adding a solution of volatile acid to the solubilized colonization factor to obtain a product in order to identify colonization factors via mass spectometry.

12. Therefore, in my opinion, the abstract does not disclose a method for identifying at least one bacterial colonization factor via mass spectrometry wherein the colonization factor is first, solubilized in 1,1,1,3,3,3-hexafluoro-2-propanol and then second, a solution of volatile acid is added to the solubilized colonization factor.

13. After reading the abstract, I would not likely be able to identify at least one bacterial colonization factor via mass spectrometry wherein the colonization factor is first dried (by evaporation or lyophilization), then solubilized in 1,1,1,3,3,3-hexafluoro-2-propanol and third, a solution of volatile acid is added to the solubilized colonization factor with a reasonable likelihood of success as the abstract teaches that the colonization factor is dissolved in a solution of hexafluoroisopropanol and acetic acid.

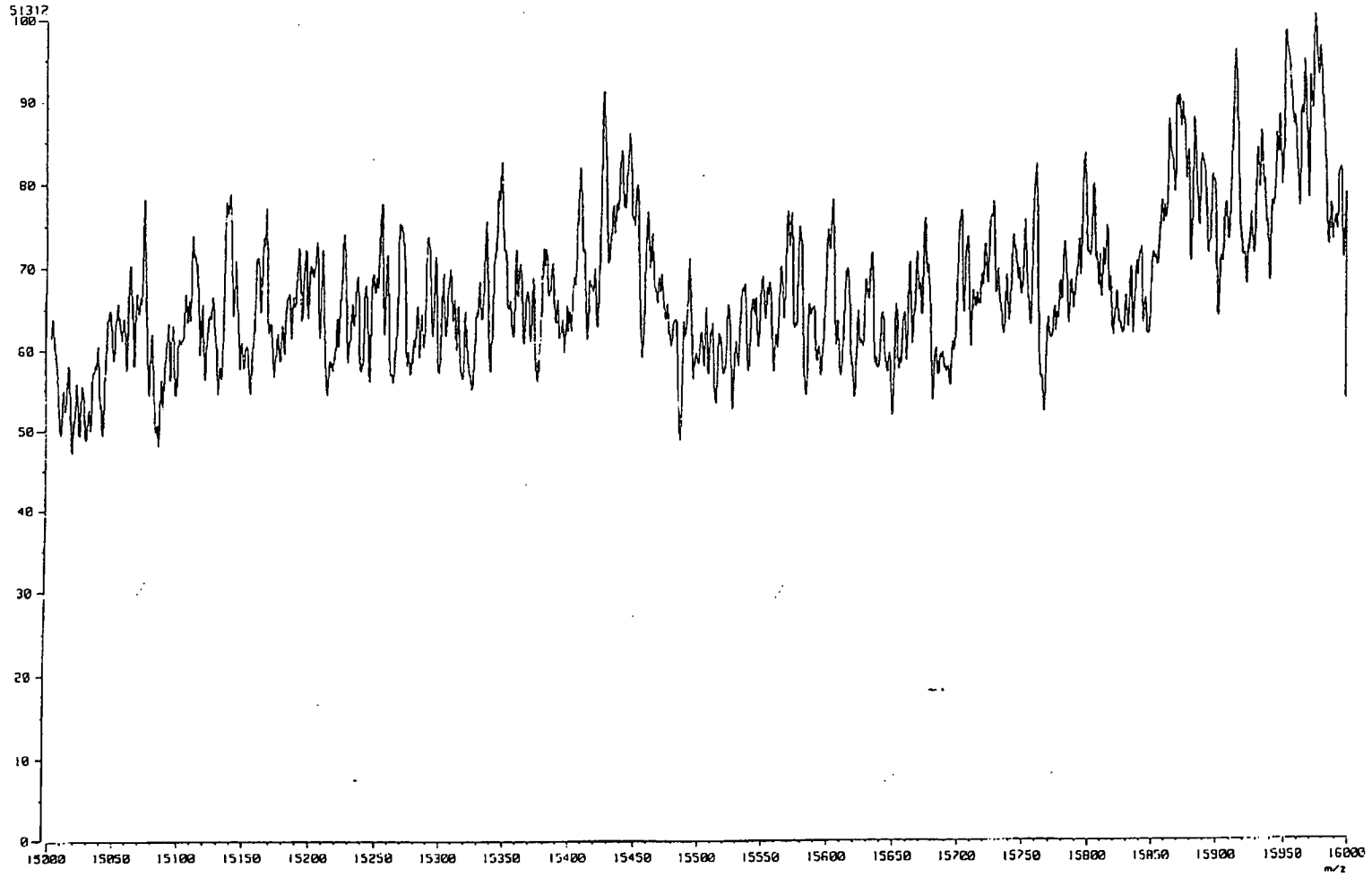
14. I hereby state that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

EXECUTED at Silver Spring this 11th day of May 2004,

By 

Ryan T. Ranallo

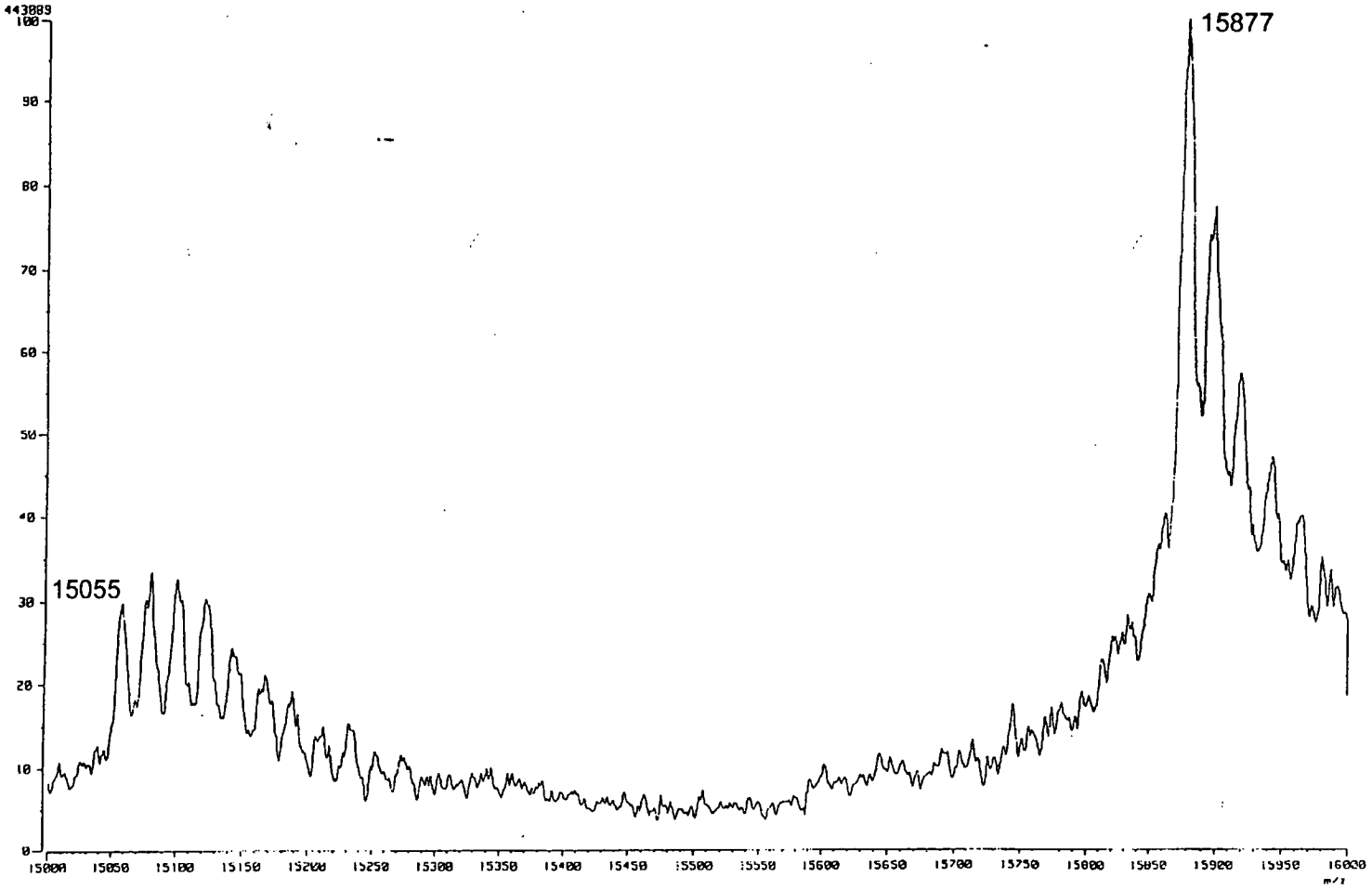
CS6 First Dissolved in 5% Acetic Acid
Followed by an Equal Volume of Hexafluoropropanol



Note: There is no sign of the 15055 and 15877 protein peaks

CS6 First Dissolved in Hexafluoropropanol

Followed by an Equal Volume of 5% Acetic Acid



Note: 1. The protein peaks are clearly seen along with their sodium adducts

2. The background (e.g. m/z 15500) is roughly constant with previous example

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Employment:

June 2002-present	Investigator Department of Enteric Infections Division of Communicable Disease and Immunology Walter Reed Army Institute of Research (WRAIR) Silver Spring, MD
2000-2002	Postdoctoral Research Fellow (CRTA) Laboratory of Molecular Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD Role of DNA Repair Proteins in Chromatin Structure

Education:

2000	Ph.D. Department of Biochemistry and Molecular Biology Colorado State University, Fort Collins, CO Dissertation Title: Functional Characterization of *Saccharomyces Cerevisiae* TFIID and TFIIA.
1996	Bachelors of Science Ohio University, Athens, OH Major: Microbiology; Minor: Chemistry

Honors and Awards:

July 2000-2002	Cancer Research Training Award (CRTA)
2000	Preecha Kownin Memorial Award for Outstanding Research by a Graduate Student, Colorado State University
1996-1999	GAAN Training Fellowship
1998	Excellence in Teaching awarded for outstanding teaching
1997	Mauricio X. Zuber Memorial Scholarship (best 1st-year graduate student)
1996	Summa Cum Laude Graduate, Ohio University

Teaching Experience:

Adjunct Faculty Johns Hopkins University 2003-present.
Instructor for Recombinant DNA Technology Laboratory. This course is part of the Masters of Science in Biotechnology in the Advanced Academic Programs in the Johns Hopkins University Krieger School of Arts and Sciences. As an instructor I was responsible design and implementation of course curriculum.

National Institutes of Health 2000-2002.
Mentor for pre-ERTA Student. Designed and supervised independent research project while a cancer research fellow at the National Cancer Institute. Responsibilities included discussing basic aspects of biochemistry and molecular biology as they related to both cancer and basic research.

Colorado State University 1997-1998.
Teaching Assistant for Undergraduate Biochemistry Courses. Teaching responsibilities included both Biochemistry lectures and designing and supervising undergraduate biochemistry laboratory experiments. During this time I developed a series of molecular biology experiments designed to introduce biochemistry students to cloning, expression and assaying recombinant proteins from *E. coli*. The experiments were incorporated into an undergraduate lab manual (Shawn O. Farrell and Ryan T. Ranallo, 1998 Experiments in Biochemistry: A Hands on Approach).

Ohio University 1994-1996.
Supplemental Instruction Leader. Responsibilities included reviewing course material during sessions held four times a week. Duties also included preparation of review session content and assisting professor in exam preparation. Courses covered included: Introduction to Zoology (Bios. 170), General Chemistry (Chem. 151), and Organic Chemistry (Chem. 301, 302).

Professional Activities and Service:

Technical Reviewer for The Department of Defense Small Business Innovation Research (SBIR) Grant. Title of Request: Innovative Manufacturing Techniques for Polysaccharide-Protein Conjugate Vaccines. September 2003.

Pending Research Proposals/Grants:

Shigella Vaccine Candidates Evaluation in Monkey Model, Co-Investigator. (Dilara Islam, Ph.D., Principal Investigator) Submitted to: NIAID for: PA-03-080.

DNA Microchip-based Technology for Genome-wide Transcriptional Profiling of *Shigella* Vaccine Strains and Host Response to *Shigella* Infection. Collaborator. (MAJ Carl Brinkley, Ph.D., Principal investigator) Submitted to: Military Infectious Disease Research Program.

Recent Training Courses:

New Faculty Workshop. "Purposeful Teaching". Karen L. Spencer, Ph.D., Purposeful Development Associates. January 2004.

"Good Laboratory Practices (GLP) Regulations: Introduction and Practical". U.S. Army Medical Research Institute of Infectious Diseases. January 2004.

Nonhuman Primate Workshop. Division of Veterinary Medicine, WRAIR. March 2003.

GLPs in the Analytical Laboratory, Shiba Associates. March 2003

Guinea Pig Handling Techniques. Division of Veterinary Medicine, WRAIR. April 2003

Rodent Handling Techniques. Division of Veterinary Medicine, WRAIR. November 2002

Animal Care and Use Program (ACUP) Orientation. Division of Veterinary Medicine, WRAIR. August 2002.

Recent Abstracts:

Ryan T. Ranallo, Antionette Hartman, Fred Cassels, and Malabi Venkatesan. Construction of Live Attenuated Shigella Vaccine Strains that express ETEC and Campylobacter Antigens. 2004. American Society of Microbiology 104th General meeting. Submitted.

Ryan T. Ranallo, Antionette Hartman, Fred Cassels, and Malabi Venkatesan. Development of Live Attenuated Shigella Vaccines that Express both (ETEC) CFA/I fimbrial protein and the B subunit of Heat-labile Toxin (LT-B). 2003. Oral Presentation. Sixth Annual Conference on Vaccine Research. Arlington VA.

Carl Wu, Paul Badenhorst, Gaku Mizuguchi, Ryan Ranallo, Xuetong Shen, Hih-Min Wang, Wei-Hua Wu, Hua Xiao. ATP-Dependent Chromatin Remodeling Complexes for Transcription. 2003. HGM 2003 Symposium.

Publications:

Xuetong Shen*, Ryan Ranallo*, Eugene Choi, and Carl Wu. 2003. Involvement of Actin-related Proteins in the Mechanism of ATP-Dependent Chromatin Remodeling. *Coauthors. *Mol. Cell.* **12**:147-155.

Xuetong Shen, Hua Xiao, Ryan Ranallo, Weihua Wu, and Carl Wu. 2003. Modulation of ATP-dependent Chromatin Remodeling Complexes by Inositol Polyphosphates. *Science* **299**:112-114.

Hua Xiao, Raphael Sandaltzopoulos, Hih-Min Wang, Ali Hamiche, Ryan Ranallo, Kyu-Min Lee, Dragony Fu, and Carl Wu. 2001. Dual Functions of the Largest NURF Subunit NURF301 in Nucleosome Sliding and Transcription Factor Interactions. *Mol. Cell.* **8**:531-543.

Susan Kraemer, Ryan T. Ranallo, Ryan C. Ogg, and Laurie A. Stargell. 2001. TFIIA interacts with TFIID via Association with TBP and TAF40. *Mol. Cell. Biol.* **21**:1737-1746.

Kathleen M. Campbell, Ryan T. Ranallo, Laurie A. Stargell, and Kevin J. Lumb. 2000. Reevaluation of Transcriptional Regulation by TATA Binding Protein Oligomerization: Predominance of Monomers. *Biochemistry.* **39**:2633-2638.

Ryan T. Ranallo, Kevin Struhl, and Laurie A. Stargell. 1999. A TATA-binding Protein Mutant Defective for TFIID Complex Formation In Vivo. *Mol. Cell. Biol.* **19**:3951-3957.

Shawn O. Farrell and Ryan T. Ranallo. 1998. Experiments in Biochemistry: A Hands on Approach. Harcourt Brace and Company, USA.

Manuscripts in Preparation:

Ryan T. Ranallo, Antionette Hartman, Fred Cassels, and Malabi Venkatesan. Development of Live Attenuated Shigella Vaccines that Express both (ETEC) CFA/I fimbrial protein and the B subunit of Heat-labile Toxin (LT-B). Manuscript in preparation.

Robinson, M.M., A. Bric, G. Yatherajam, R.T. Ranallo, A. Borland, M.R. Paule and L.A. Stargell. TAF11 Interacts with TFIIA via Two Distinct Domains to Promote Stable Association of TFIIA-TBP-DNA Complexes. Manuscript in preparation.